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Definitive Molecular Cytogenetic Characterization of 15 Colorectal Cancer Cell Lines

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In defining the genetic profiles in cancer, cytogenetically aberrant cell lines derived from primary tumors are important tools for the study of carcinogenesis. Here, we present the results of a comprehensive investigation of 15 established colorectal cancer cell lines using spectral karyotyping (SKY), fluorescence in situ hybridization, and comparative genomic hybridization (CGH). Detailed karyotypic analysis by SKY on five of the lines (P53HCT116, T84, NCI-H508, NCI-H716, and SK-CO-1) is described here for the first time. The five lines with karyotypes in the diploid range and that are characterized by defects in DNA mismatch repair had a mean of 4.8 chromosomal abnormalities per line, whereas the 10 aneuploid lines exhibited complex karyotypes and a mean of 30 chromosomal abnormalities. Of the 150 clonal translocations, only eight were balanced and none were recurrent among the lines. We also reviewed the karyotypes of 345 cases of adenocarcinoma of the large intestine listed in the Mitelman Database of Chromosome Aberrations in Cancer. The types of abnormalities observed in the cell lines reflected those seen in primary tumors: there were no recurrent translocations in either tumors or cell lines; isochromosomes were the most common recurrent abnormalities; and breakpoints occurred most frequently at the centromeric/pericentromeric and telomere regions. Of the genomic imbalances detected by array CGH, 87% correlated with chromosome aberrations observed in the SKY studies. The fact that chromosome abnormalities predominantly result in copy number changes rather than specific chromosome or gene fusions suggests that this may be the major mechanism leading to carcinogenesis in colorectal cancer. Published 2009 Wiley-Liss, Inc.†

INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies in many parts of the world and a leading cause of cancer deaths in both men and women (Jemal et al., 2008). The studies of the adenoma-carcinoma sequence have made it possible to compare all stages of colorectal carcinogenesis (Ried et al., 1996; Bardi et al., 1997), and the studies of CRC cell lines provide valuable information about the genomic instability associated with this type of cancer (Melcher et al., 2000, 2002; Abdel-Rahman et al., 2001; Tsushimi et al., 2001; Kawai et al., 2002; Kuechler et al., 2003; Roschke et al., 2003; Camps et al., 2004a,b; Kleivi et al., 2004). CRC cells exhibit two types of genetic instability: mismatch repair deficiency (MMR^-) leads to microsatellite instability at the nucleotide level and results in base substitutions or deletions, or insertions of a few nucleotides, whereas MMR^+ tumors exhibit defects in chromosome segregation, leading to both numerical and structural chromosome abnormalities (Lengauer et al., 1997; Ghadimi et al., 2000; Rowan et al., 2000; Gayet et al., 2001). MMR^- CRC cell lines are near-diploid and chromosomally stable and represent about 15% of all CRC, whereas the MMR^+ lines are highly aneuploid. In studies presented by the Sanger Institutes Cancer Genome Project (www.sanger.ac.uk), five specific markers (*BAT25*, *BAT26*, *D5S346*, *D2S123*, and *D17S250*) were used to characterize the microsatellite stability of CRC tumors and cells lines. Those studies, which included 13 of our 15 cell lines, confirmed their MMR status.

In this study, we analyzed the karyotypic patterns of 15 cell lines applying a combination of spectral karyotyping (SKY), fluorescence in situ hybridization (FISH), and comparative genomic hybridization (CGH) techniques, and compared our results with SKY/M-FISH and CGH studies

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previously reported for a subset of these cell lines; five of the cell lines have not been previously characterized by SKY or M-FISH. We also compared our results with 345 cases of adenocarcinoma of the large intestine listed online in the Mitelman Database of Chromosome Aberrations in Cancer. A companion study using array CGH (aCGH) and array-based global gene expression profiling in these cell lines appears in a separate publication (Camps et al., 2009).

MATERIALS AND METHODS

Cell Lines

Fifteen CRC cell lines were included in this study. Five of the cell lines had a near-diploid karyotype and were known to exhibit MMR^- (defects in DNA mismatch repair): DLD1, HCT116, p53HCT116, SW48, and LoVo. Ten aneuploid cell lines lacked the MMR^- phenotype: HT-29, SW480, SW620, SW837, LS411N, COLO320DM, T84, NCI-H508, NCI-H716, and SK-CO-1. SW480 and SW620 were derived from the same patient: SW480 originated from a primary tumor and SW620 from a metastatic lymph node in the abdominal wall a year later. All of the cell lines were obtained from the American Type Culture Collection (ATCC) with the exception of p53HCT116, a derivative of HCT116 with a mutation in *TP53* (Bunz et al., 1998), which was kindly provided by Curtis C. Harris of the National Cancer Institute, NIH.

SKY, FISH, and CGH

SKY was performed for the identification of chromosomal abnormalities according to the technique of Schröck et al. (1996); the protocols are available on the Ried laboratory's web site at http://www.riedlab.nci.nih.gov/protocols.asp. A minimum of 10 SKY and DAPI metaphase images were acquired and analyzed for each cell line. The karyotypic and FISH findings were described in accordance with the ISCN nomenclature rules (ISCN, 2005).

CGH with cell line DNA and normal control genomic DNA was performed on nine of the cell lines using normal sex-matched metaphase chromosome slide preparations, according to a modification of the method described by du Manoir et al. (1993); protocol details are available at http://www.riedlab.nci.nih.gov/protocols.asp. Average ratio profiles were calculated from 11 to 15 images per cell line.

FISH studies were conducted using bacterial artificial chromosome (BAC) clones, unique sequence probes, centromere probes, and whole-chromosome paints generated in-house using standard nick translation or labeling PCR techniques.

Cancer Chromosomes Database Analysis

The NCI/NCBI Cancer Chromosomes database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=cancerchromosomes) (Knutsen et al., 2005), which is part of the NCBI Entrez system (which includes PubMed), contains the public cases in the SKY Database and all 54,000+ cases in the Mitelman Database of Chromosome Aberrations in Cancer (Mitelman et al., 2008; http://cgap.nci.nih.gov/Chromosomes/Mitelman). Cancer Chromosomes contains several tools for analyzing data, including searching of multiple types of aberration data. Karyotypic, SKY/M-FISH, and CGH data can be searched seamlessly based on the underlying cytogenetic features of the aberrations they demonstrate. It also contains the ability to find similar cases based on textual content. The Similarity Report tool, which compares cytogenetic abnormalities involving chromosome breakpoints, junctions (fusion sites of translocations, inversions, and insertions), numerical and structural abnormalities by chromosome, and bands gained or lost, was used to analyze breakpoints and junctions in 345 cases of adenocarcinoma of the large intestine in the Mitelman Database. Data on the recurrent abnormalities in these cases were extracted from the Mitelman Database itself.

RESULTS

SKY and FISH

The results of the SKY and FISH studies are presented in Table 1, together with a comparison of the results of SKY/M-FISH studies in the literature. All of the cell lines analyzed in this study were entered into the NCI/NCBI online SKY/M-FISH and CGH Database (http://www.ncbi.nlm.nih.gov/sky/skyweb.cgi; Knutsen et al., 2005) (see the "Access to Public SKY/M-FISH and CGH database" link, submitter T. Ried). The Database presents the written karyotype, a "SKYGRAM" (colored ideogram depicting the abnormal karyotype), the CGH profile, case details and cell line information, and the results of other laboratory studies such as FISH.

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TABLE 1. SKY Karyotypes of 15 Colorectal Cancer Cell Lines and Comparisons with SKY/M-FISH Studies in the Literature

Cell line	SKY karyotype Ried lab	Ref. (see footnote)	Abnormalities divergent in other studies [ref #]
DLD1 (MMR$^-$)	46,XY,**dup(2)**(p14p22)[6],**der(6)t(6;11)**(p25;p15.3)[6][cp6].ish der(6)t(6;11)(wcp1−,wcp6+,wcp11+)	1, 2, 4	+dup(1)(1p?)[1],−dup(2)[2],−der(6)[2],+der(16)(t(7;16)[4]
HCT116 (MMR$^-$)	45,X,−**Y**[10],**der(4)t(4;17)**(q3?;?q21)[10],del(9)(q11)[2],**der(10)dup(10)**(q23.1q26.1)**t(10;16)**(q26.1;q23)[10],**der(16)t(8;16)(q13;pter)**[10],**der(18)(:4q3?::17q?22→17q21.3::18pter→18qter)**[10][cp10].ish −Y(wcpY−),der(10)(wcp10+,wcp16+),der(4)t(4;17)(wcp4+,wcp17+),der(18)t(4;17)t(17;18)(wcp4+, wcp17+,wcp18+)/45–46,idem,−der(4),+der(18)t(17;18)(q21.3;pter) [cp7].ish −Y(wcpY−),der(18)t(17;18)(wcp4−,wcp17+,wcp18+)	1, 3a, 3b, 4, 5, 9	−der(4)t(4;17)[1,3a,3b,4,9],−dup(10)(q24q26)t(10;16)[3a,3b,5,9]+der(10)dup(10)t(10;12)[5],no chr 4 in der(18)t(17;18)[1,3a,3b,4,9],+der(21)t(11;21)(q14;p13)[3b]
P53HCT116 (MMR$^-$)	45,X,−Y[18],t(5;7)(q13;pter)[18],der(10)dup(10)(q23.1q26.1)t(10;16)(q26.1;q23)[18],der(16)t(8;16)(q13;pter)[18],der(18)t(17;18)(q21.3;pter)[18][cp18]	None	
SW48 (MMR$^-$)	47,XX,+**7**[19],**dup(10)**(q21.3q23.1)[20],+12[3],−18[3],**der(22)t(14;22)**(q12;qter)[19][cp20]	3a	no +12 or −18 [3a]
LoVo (MMR$^-$)	49(47–50),XY,**t(2;12)**(q22;p12.1)[20],+**5**[19],+**7**[20],+**12**[19], **i(15)(q10)**[20][cp20]	1, 2, 3a, 3b, 4, 9	+del(2)(?)[2],+t(7;18)(q31.3.;q22)[3b],+t(11;14)(p14;q21)[3b], no+12[2,3b,9],+15[3b),−i(15)(q10)[3b]
HT-29	68–70(67–71),XX,**del(X)(p11.4)**[19],**del(3)**(p21)[11],**der(3)ins(3;12)**(p12;?)[16],+der(3)del(3)(p25)ins(3;12)(p12;?)[5],+**der(3)t(X;3)**(q28;qter)[11],+**i(3)(q10)**[21],**del(4)**(q31.3)[21],+**der(5)t(5;6)**(q11.1;q25)[10], **del(6)**(q14)[9],**t(6;14)**(q21;q13)[15],**der(6)t(6;14)**(q21;q13)[5],+**del(7)(p15)**[17],−**8**[20],**der(8)i(8)**(qter→q10::q10→q24::**hsr**::q24→qter)[21],+**11**[20],**ider(13)(q10)del(13)(q14)**[11],der(13)t(5;13)(p13;p11.1)[12],+**i(13)(q10)**[18],−**14**[21],−**14**[4],+**15**[20],**der(17)t(17;19)** (p11.1;q12)[21],**i(18)(p10)**[19],−**19**[13],**ider(19)(q10)dup(19)**(q13.1q13.4)[19],+i(20)(q10)[21],−**21**[[21],**der(22)**(:17q21→17q11.2::22q12→22cen→22q12::17q11.2→17q21)[20][cp21].ish der(3)ins(3;12)(wcp3+,wcp12+),der(3)t(X;3)(wcpX+,wcp3+),i(8q)(ampMYC+)	1, 3a, 4, 5, 7, 8	−del(X)[3a],+del(1)(p35)[8],+der(2)t(1;2)(q32;q11)[1,8][a],+der(2)t(2;22) (q36;?)[5],−del(3)[1,3a,4,5,8],−der(3)t(X;3)[1,3a,5,7,8],+der(3)t(3;5)(p21;p?)[3a],−der(3)ins(3;12)[1,3a,5,7],+der(3)del(3)(p14?)del(3)(p25?)[7], +der(3)del(3)(p14)t(3;21)(q28;?)[5],−i(3)(q10)[1,4,5,7],−del(4)[8],+der(4)t(2;4)(q35;q11)[8],+del(5)(q11)[4,5,7],−der(5)t(5;6)[3a,4,5,7],+der(5)t(5; 19) (q11;q11)[8],−6[1,3a,4,8],−del(6)[1,3a,4,8],−t(6;14)[1,8],+der(6)t (6;9) [1,8][a], −del(7)[1,3a],no−8[1,3a,4,5,7],+der(9)ins(9;?)[4,?7]],+der(?)t(9;11)[1], no+11[4,7,8],+del(11)(p13)[8],+der(11)t(11;13)[1],+der(?)t(11;16)(q10; ?p10)[1],+der(11)t(11;20)[8],no−13[3a,7],der(13)i(13)[1,3a,4,5, 8],+der(13)i(13)t(5;13)(q31;q21)[9],+der(13)t(7;13)(?;q10)[1],no−14[7], no+15[7,8],+der(15)t(15;15)(p13;?)[3a],+del(16)(q13)[8],−17[5,8],+del(17)(p11)[3a],−der(17)t(17;19)[3a],no−19[1,3a,5,7],−i(19)dup(19)[1,3a,8], +20[1,3a,4,8],+der(20)dup(20)(q?12q?13)[5],no−21[7],−der(22)t(17;22) [3a,4,7], +ider(22)(p10)ins(22;22)[7]

(Continued)

TABLE 1. SKY Karyotypes of 15 Colorectal Cancer Cell Lines and Comparisons with SKY/M-FISH Studies in the Literature (Continued)

Cell line	SKY karyotype Ried lab	Ref. (see footnote)	Abnormalities divergent in other studies [ref #]
SW480	57–58(54–61),XX,−**Y**[7],**t(1;9)**(q12;q11)[7], **+der(2)t(2;12)**(q22;q12)[7],**der(3)**(qter→q25::p22→qter)[7],**t(5;20)**(q15;p12)[7],+**der (7)t(7;13)**(q34;q21)[5],+**der(7)inv(7)(q34q22)t(7;14)**(q22;q24)[7],**der(8)t(8;19)**(q11.2;?q12)[7], **der(9)t(8;9)**(q11.2;p12)[7],**der(10)t(3;10)**(q11.1;p11.2)[b][7],+**11**[6],der(12)(3pter→3p21::12p11.2→q11:) [6], **i(12)(p10)**[7],+**13**[6],+**17**[6],der(18)t(18;20)(q12;?)[7], +der(18)t(18;20)(q12;?)[6],**der(19)t(8;19)**(?;q13.1)**t(8;19)**(?;?)**t(5;19)**(?q33;?)[7],+**20**[3],+**der(20)t(5;20)**(q15;p12)[6], +21[7][cp7].ish t(5;20)(wcp5+,wcp20+),i(12)(p10)(KRAS+),der(12)t(3;12)(KRAS+),der(19)(wcp5+,wcp8+,wcp19+,ampMYC+), der(18)(wcp18+,wcp20+)/55–66,idem,−der(3),−der(7),+i(7)(q10),+der(8)t(2;8)(q24orq32;pter),+der(9)t(8;9)(q13;p13)t(9;14)(q34;q22),−der(10)t(3;10)(q11.1;p11.2), +der(10)t(3;10)(q21;p11.2),−der(12),+13,+der(17)t(5;17)(?;p11.2)[cp2].ish t(5;20)(wcp5+,wcp20+),i(12)(p10)(KRAS+),der(19)(wcp5+,wcp8+,wcp19+,ampMYC+),der(18)(wcp18+,wcp20+)	1, 3a, 4, 6, 10	+der(X)t(X;15)(q22;q?)[3a],+del(2)[1],+der(2)t(2;18)[1],+del(3)(q11∼12) [1,4,6,10],−der(3)[1,4,6,10], +der(3)t(3;10)(p24;p12)[6],+del(5)[6], +der(?)t(5;12)[1], +der(5)t(5;20)(q11;?)[10],−t(5;20)[6],+der(7)t(1;7)[1],−der(7)t(7;13)[3a,6],+der(7)dup(7)[6],+der(8)t(5;8)[6], −der(8)t(8;19)[3a,6],+der(10)t(X;10)[3a],+der(10)t(3;12;10)[b][1,4,6],+der(10)t(3;10)(p21;p13)[b][10],+der(10)t(10;15)[1], no+11[4,10],+der(11)t(11;15)[1],+del(12)(q13)[1,4,10],+der(12)t(14;14)[1],−i(12)(p10)[1,4,6,10],no+13 [4,6,10], +der(13)t(3;13)(p23;q21)[6],+dic(15;18)(15per→15q11::18p11→18q21.1)[6],no+17[1,4,10],+del(17)(q?)[1],+del(18)(q12)[1,4,11], +der(18)add(18)(p?)del(18)(q12)[4], +i(18)(p10)[3a,6],+der(19)t(6;19)(q22;q13.1)[6],−der(19)[6],no+20[3a,4,6,10],−der(20t(5;20)[1,10],no+21[4,6,10],+22[3a]
SW620	50(46–57)<2n>,XX,−**Y**[11],**der(2)t(2;12)**(p24.1;?p11.2)[10], **del(3)**(p14)[11],**del(4)**(q31.1)[11],**t(5;20)**(q15;p12)[10],+**der(5)t(5;20)**(q15;p12)[10],+6[2],**der(6)t(6;7)**(q25;q32)[10],+7[4], **del(7)(q22)** [3],**der(7)del(7)**(p11.2)**del(7)**(q21.2)[10], **der(8)t(8;13)**(p23;q14.3)[11],**der(8)t(8;17)**(p23;q25)[10], **der(10)t(10;13)**(q23;q14.3)[9],+**11**[8],+del(12)(q11)[2], −13[9],**der(16)(3**qter→3q21::hsr**16**::**8**?::hsr**16**::**10**q23.1→10qter)[11],**der(18)t(15;18)(p11.1;q21)t(15;17)(p13;q22)**[10],−22[4], +der(22)t(2;22)(?;p11.2)[4][cp11]	1, 5, 10	+der(X)t(X;6)[5,10),+der(?)t(X;18)[1],+del(5)[1],+der(5)t(5;7)(q11;p11)[5], −del(7)[5],−8[1],no+11[10],no−13[5],−der(16)[1],+der(16)dup(16)t(3;16)t(6;16)[1],−der(18)t(15;18)t(15;17)[1],+der(?)t(5;18)[1],no−22[1]
SW837	40(37–40),**der(X)t(X;5)**(qter;q13)[14],−**Y**[14],**der(1)t(1;8)**(p12;p12)[13],**der(3)t(3;11)**(p12;q13.4)[14],**del(6)**(q14q22.3)[13],**der(7)t(7;19)**(qter;?q12)[14],**dic(8;17)**(p12;p11.2)[13], **der(11)t(1;11)**(p32;p14) [12],der(11)t(11;14)(pter;q24)[13],−**13**[14],**der(13)t(13;15)**(q10;q10)[14],−**17**[14],−**18**[14],−**19**[14],i(20)(q10)[14], del(22)(q11.2)[14][cp14]/39–40, idem,−der(X)t(X;5),+der(X)t(X;8) (qter;q22)[3][cp3]/40,idem, +X[3],−der(X)t(X;5)[cp3]	1	+del(1),+(2)t(2;17),+der(7)t(2;7),−der(11)t(11;14),+t(16;20)
LS411N	72(70–75)<3n>,X,der(X)t(X;14)(p22.1;q11.2)[6],−**Y**[8],+**del(3)**(p13)[5],+der(4)(15qter→15q21.2::13q?12.2→13q11::4p12→4qter)[2], +der(5)(5pter→5q11.2::13q?12.1→13q?12.2::8?q24.1→8?qter)[2],+i(5)(p10)[8],der(6)t(6;15)(q23.1;q21.2)[7],i(6)(p10)[5],i(6)(q10)	1	−der(X)t(X;14),+der(X)dup(X)t(X;5),+del(1),−der(4), +del(5),−der(5),−i(5)(p10),+del(6),+der(6)t(5;6),+del(6), −der(6)t(6;15),−i(6)(p10),+dup(6), −i(6)(q10),−del(7),−der(7;8),−der(7)t(7;15),+dup(7),−der(8)t(7;8),−der(8)t(8;12),

(Continued)

TABLE 1. SKY Karyotypes of 15 Colorectal Cancer Cell Lines and Comparisons with SKY/M-FISH Studies in the Literature (Continued)

Cell line	SKY karyotype Ried lab	Ref. (see footnote)	Abnormalities divergent in other studies [ref #]
	[8],del(7)(q22.1)[6],der(7;8)(p13;q11.1)[7],+**der(7)(7pter→7q?::7hsr::12q21.2→12qter)**[5],+der(7)t(7;15)(p13;q21.2)[2],der(8)t(7;8)(p15;q24.23)[6],+der(8)t(8;12)(p11.2;q21.2)[2],+i(12)(p10)[5],der(13)(15qter→15q21.2::13q22→13q11::13p11.2→13qter)[2],+**der(13)t(13;13)**(p11.2;q11)del(13)(q22)x2[6],−14[8],der(14)t(5;14)(q15;q13)[5],**i(14)**(q10)[7],+der(17)t(7;17)(17pter→17q11.2::7?)[5],**ace(18)**[7],+ace(18)[3],−21[8],i(21)(q10)[6][cp11]		+der(?)t(8;22),+del(9),+der(?)t(10;17),+del(11)(q?),+del(12),−i(12)(p10),−der(13)t(13;15),−der(14),+del(17),−der(17)t(7;17),+dup(19)(p?),−i(21)(q10),+der(?)t(12;21),der(?)t(6;22)
COLO320DM	52<2n>,XX,**t(1;5)(q10;p10)**[7],t(1;16)(p36.21;p13.1)[8],**del(2)**(q21.3)[8],**der(2)t(2;11)**(p22.1;p13)[7],+der(2)(2pter→2p21::13q12::8q24.21::2p21→2q31::13q12::8q24.21::2q31→2qter)[5],der(3;9)(q10;p10)del(9)(p13.3)[7],+der(4)(13q12::8q24.21::4p16.3→4q21.21::7q22.3→7q36.1::16p11.2→16pter)[7],**der(5)**(3qter→3q22.1::13q12::8q24.21::1p13.3→1p11.2::5p12→5qter)[7],der(1;5)(5pter→5p10::1q10→1q23.1::13q12::8q24.21→8qter)[7],**t(6;7)**(q13;q22.3)dup(6p25→6p23)[8],t(6;7)(q13;q22.3)[7],der(8)t(8;17)(q24.3;q21.31)[6],der(9;15)(q10;q10)[7],+**der(9)t(3;9)**(q12;p21)[8],+del(12)(12q13.3q22)[8],**der(13;13)**(qter→q21::q21→q10::q10→qter)[8],der(1;14)(13qter→13q12.1::1p13.3→1q12::14p11.2→14qter)[8],−15[7],+16[7],der(17)t(2;17)(q11.2;p11.2)[7],+**20**[7],−21[7],+del(22)(q11.23)[6],50∼100**dmin**[8][cp8].ish der(2)t(2;13;8;2;13;8;2)(CDX2+,MYC+),der(4)t(13;8;4;7;16)(CDX2+,MYC+),der(5)t(3;13;8;1;5)(CDX2+,MYC+),der(1;5)t(5;1;13;8)(CDX2+,MYC+), der(8)(MYC+),der(13;13)(CDX2+),der(1;14)t(13;1;14)(CDX2+), dmin(MYC+,CDX2+)/54–57,idem,+der(X)t(X;20)(p22.1;q13.3),−del(2),+der(3)t(3;?)(q12;?),+4,−der(4),−der(5),−der(1;5)(3;13;8;1;5), +der(1;5)(5pter→5p10::1q10→1q21.1::13q12.1→13qter),+der(5) (7pter→7p13::1p22→1p12::5p12→5qter),−t(6;7),−t(6;7)dup(6p),+del(7)(p13),−der(8),+der(10)t(10pter→10q24::13q12::8q24.21::3q11.2→3qter),−13,−der(1;14),+i(14)(q10),+16,+der(17)t(1;17)(p12;p11.2)del(1)(p13),−der(17)t(2;17),+del(20)(q13.3),+21,+22[cp3]/37–53,idem,−der(4),+der(4)(4p16.3−>4q21.21::13q12::8q24.21::14q11.2→14qter),−der(1;5)t(5;1;13;8),+der(1;5)(5pter→5p10::1q10→1q23.1::13q12→13qter),−der(1;14),+i(14)(q10),+der(16)(1pter→1p36.21::16p13.1→16q22::22q11.2→22qter),+der(19)(19pter→19q11::hsr8/13::?16),−dmin[cp4]/54–55,idem,−X,+der(4)t(4;13;8;14),−der(1;5)t(5;1;13;8),+der(1;5)(5pter→5p10::1q10→1q23.1::13q12→13qter)x2,−t(6;7),+der(7)(14qter→14q11.2::7p22→7q22.3::6q13→6qter),+der(8)t(1;8)(p36.3;q24.3),+der(8)(:8p12→8q11.2::hsr8/13),+der(12)t(12;20)(p11.2;p11.2),−14,+der(17) t(1;17)(:1p13→1p12::17p11.2→17qter),+der(19)(19pter→19q11::hsr8/13::?16),−dmin[cp2]	2, 3a	+der(X)t(X;4)(p11;q33)[3a],−del(2)[3a],+der(2)(?)[3a],−der(2)t(2;11)[3a],+der(2)t(2;17)(p24;q21)[3a],+ider(2)(p10)ins(2;8)(p22;q24)[2],+del(3)[2],+der(3)t(3;?15)[3a],+4[2],+der(4)t(3;4)[2],−der(5)t(3;5)[3a],+der(5)t(5;7)(p15;p15)[3a],−t(6;7)[3a],+7[2],+der(7)t(2;7)(p23;p21),+der(7)t(7;8)[2], +der(8)t(8;13)(p24;?)[3a],+9[2],+del(9)[3a],−der(9)t(3;9)[3a],+der(9) t(9;12)(p13;p11)[3a],+10[2],+der(10)t(3;10)(?;q26)[3a],+ins(10;8)(q24;q24)[2],−i(13)[3a],+ider(13)(q10)t(8;13)[2,3a],−i(14)(q10)[2],+der(14;14)(q10;q10)t(13;14)(q20;q10)[2],+der(15)t(9;15)[2],+der(15)t(9;15)(?;p11t(3;15)(?;q?) [3a],+der(16)t(5;16)(p11;p11)t(13;16)(q11;q22)[3a],+der(16)t(2;7;8;16)(?;?;?;p13)[3a],−17[2],+der(17)t(17;18) [3a],no +20[3a],+der(21)t(5;21)[2],−dmin[3a]

(Continued)

TABLE 1. SKY Karyotypes of 15 Colorectal Cancer Cell Lines and Comparisons with SKY/M-FISH Studies in the Literature (Continued)

Cell line	SKY karyotype Ried lab	Ref. (see footnote)	Abnormalities divergent in other studies [ref #]
T84	57(53–58),X[2],i(X)(q10)[5],−Y[7],+der(2)t(2;17)(pter;q21) del(2)(q21q24)[7],?del(3)(p25p25)[7],+der(3)(17qter→17q11.2::1q25→ 1q21::3p22→3p21::8q22→8q24::3p21→3qter)[7],der(4)t(4;7) (q31;q36)[7],der(6)t(2;6)(q37;qter)[6],dup(6)(q22qter)[6],+der(7) t(X;7)(q13;q22)[6],+der(7)t(3;7)(q26;qter)[7],?del(9)(p13p13) [7],+der(10)del(10)(p14)del(10)(q25)[6],+11[4],+11[2],+der(11) dup(11)(p14p12)t(7;11)(q11.2;pter)[1],+12[7],+12[6],−13[7],+der (13)t(X;13)(?;p11.2)del(13)(q?14)[3],i(13)(q10)[7],+der(15)t(12;15) (?;p11.1)[7],+der(15)t(12;15)(?;p11.1)[1],+der(16)t(11;16)(p11.2;qter) [7],der(17)t(1;17)(q21;q23)t(1;5)(q24;q21)[7],−18[3],der(18)t(X;18) (p21.1;p11.1)del(18)(q21)[4],+19[1],+del(20)(p11.2)[7],+der(20)t(14;20) (q11.2;qter)[7],der(22)t(X;22)(p11.2;p11.2)del(X)(p21)[7],der(22) t(X;22)(p11.2;p11.1) [7][cp7].ishder(2)(wcp17+),der(3)(wcp1+,wcp17+), der(7)(wcpX+),del(9)(RP1157914+[9p21]),der(13)(wcpX+),der(15) (wcp12+,wcp15+,cep12−),der(17)(wcp1+,wcp5+,EGR1+[5q31], D5S23−[5p15.2],wcp17+),der(18)(wcpX+),der(22)x2(wcpX+,wcp22+)/ 58–59(51–60),idem,−der(3)t(3;8)t(3;8)t(1;3)t(1;17),+der(3)t(3;8) (p23;q21.2),−der(4),+del(7)(q11.1),−11,−12,+19[cp6].ish der(3) (wcp1−,wcp17−)	None	
NCI-H508	98<4n>,XX,−Y[8],−Y[7],idel(1)(q25.1→q10::q10→q25.1)[8], +2[6],+der(3;13)(q10;q10)[9],+der(3;13)(q10;q10)[6],+der(3;13) (q10;q10)[1],−4[7],del(5)(q14.2q33.3)[9],+del(5)(q14.2q33.3)[8], der(6)t(3;6)(q27;q27)[8],+der(6)t(6;?10 or ?12)(q27;?)[6],+7[7], +7[4],+8[2],+9[3],+del(9)(?q11)[4],+del(9)(?q11)[3],+11[3],−12[3], +13[8],+13[8],+13[8],+13[6],der(15;20)(q10;q10)x2[9],i(17)(q10) [9],+i(17)(q10)[4],−19[3],−21[3],+21[4],+21[3],+21[1],>3dmin [9][cp9].ish dmin(wcp14+)	None	
NCI-H716	58(49–61)<3n>,X,−Y[14],der(X)t(X;20)(q11.2;q11.2)[12],+der(X)t (X;20) (q11.2;q11.2)[5],−2[3],del(2)(2q12q21)[12],der(2)t(2;3) (q37.3;q24)[9],−3[14],−4[14],−5[5],der(5;7)(p10;p10)[13],+der(5) t(5;13)(q31.1;q32.1)[11],−6[11],−6[3],+t(7;15)(q21.12;q22.1)[14], −8[14],−9[14],−9[3],der(9)t(4;9)(q12;p13.3)[10],der(10)t(10;14) (q21.2;q12)[13],+i(10)(p10)[7],−11[14],der(11)t(2;11)(p11.2;q21)[13], +13[2],der(13)t(X;13)(p11.1;p11.1)[11],der(13)t(20qter→ 20q11.2::13q14.1→13q10::13q10→13q22:)[13],−14[6],−15[3], −16[5],+der(16)t(11;16)(p11.2;p12.2)[13],−17[14],−17[7], −18[11],−18[3],−19[14],−19[3],+20[3],del(20) (p11.21)[11],der(20)(:p11.21→q11.21:)[11],−21[14],−21[13],i(21) (q10)[8],−22[14],der(20;22)(q10;q10)[11],∼40dmin[14][cp14] .ish dmin(MYCx∼20)[14],dmin(RP11–62L18x∼20)(10q25.1)[14]	None	

(Continued)

TABLE 1. SKY Karyotypes of 15 Colorectal Cancer Cell Lines and Comparisons with SKY/M-FISH Studies in the Literature (Continued)

Cell line	SKY karyotype Ried lab	Ref. (see footnote)	Abnormalities divergent in other studies [ref #]
SK-CO-1	70(55–74)<3n>,X,del(X)(q22)[4],dic(Y;11)(11pter→11q13.5::Yqter→Yq11.1::Yq11.23→Yqter)[6],del(1)(p33)[5],der(1)t(1;5)(q10;p10)del(5)(p14)[2],+der(1)(7qter→7q11.2::5p15→5p12::1p12→1qter)[5],+der(1)t(1;7)(q11;q22)[5],+der(1)(:1p33→1q11::17q11.2→17qter::5p13.3→5pter)[6],+dic(1;9)(9qter→9q11::1q11→1qter)[3],der(2)(:p21→q21::q23→qter)[6],dic(2;17)(8qter→8q22.3::17qter→17q11.1::2q11.1→2qter)[6],+dic(2;11)(:2q31::2q11.1::18hsr::11q12.2→11q14::2q31→2qter)[5],del(3)(p25)[6],del(3)(p22p21.3)[6],del(4)(p16)[6],der(4)t(4;18)(p16.1;p11.2)[4],del(5)(p14)[5],−6[4],del(6)(p23)[6],+der(6)(hsr6?p::pter→qter)[2],del(7)(q22)[6],+del(7)(q22)[2],der(7)(X;7)(p21;p21)[4],del(8)(p21)[6],i(8)(q10)[6],del(9)(q22q34)x3[4],del(9)(q22q34)x2[2],+der(9)(9pter→9q12::11q12→11q14:)[5],del(10)(p11.2)[4],der(10;16)(q10;p10)[6],del(11)(q24)[5],der(11)(:q13.4→q23.3::pter→q23:)[2],del(12)(p12)[6],der(12;18)(p10;p10)[5],+der(12)(:13q13→13q12.1::12q11→12q13.2::14q11.2→14qter)[2],+dic(12;12)(qter→q11::p12→qter)[6],del(13)(q12q13.3)[4],der(10;13)(q10;p10)[4],dic(13;14)(8qter→8q23::14q22→14q11.1::13q11→13qter)[6],+dic(13;14) (:14q22→14q11.1::13q11→13qter)[5],del(14)(q24)[4],i(14)(q10)[6],−15[3],del(15)(q13.3q22)[4],der(6;15)(p10;q10)[2],−16[6],del(16)(q21)[5],min(17)[6],der(7;18)(:18p11.2→18p10::7p10→7p15:)[5],der(18)(7?q::11q13.1→11q21::18pter→18qter)[6],+i(18)(p10)[6],−20[6],der(20)(15qter→15q22.3::7::20pter→20q11.2::hsr20q)[4],del(21)(q11.2q21)[6],del(22)(q13)[6],der(22)(22pter→22q11.2::hsr22(?22q11.2→22q12)::17q22→17qter)[4][cp6]/72–73(54–76)<3n>,idem−der(1)(7,5,1),+der(5;18)(p10;q10),−der(6),−der(6;15)[cp11]	None	

The aberrations in the currently studied cell lines that are shared with those in other publications in the literature are in boldface; only chromosomes involved, not breakpoints, are in bold since interpretations of bands involved may be somewhat variable.

References: 1. Abdel-Rahman et al., 2001; 2. Tsushimi et al., 2001; 3a. Kleivi et al., 2004 (Radium Hospital); 3b. Kleivi et al., 2004 (INSERM); 4. Camps et al., 2004a; 5. Roschke et al., 2003; 6. Camps et al., 2004b; 7. Kawai et al., 2002; 8. Kuechler et al., 2003; 9. Melcher et al., 2002; 10. Melcher et al., 2000.

[a]Seen in one cell in Ried's study.

[b]All der(10)t(3;10) and der(10)t(3;12;10) most likely same marker: Camps et al. (2004b) and Abdel-Rahman et al. (2001) found small insertion of chromosome 12.

Molecular karyotypes have not been reported previously for five of the lines: p53HCT116, T84, NCI-H508, NCI-H716, and SK-CO-1. Of the 10 lines reported in the literature, each had been studied by one to six investigative groups (Melcher et al., 2000, 2002; Abdel-Rahman et al., 2001; Tsushimi et al., 2001; Kawai et al., 2002; Kuechler et al., 2003; Roschke et al., 2003; Camps et al., 2004a,b; Kleivi et al., 2004). Abnormality divergence from other studies, either as absent or additional aberrations, is shown in the last column of Table 1, together with the relevant reference designation in brackets.

Eight cell lines were cytogenetically quite similar from one study to another, including four diploid lines (DLD1, HCT116, SW48, and LoVo) and four aneuploid lines (HT-29, SW480, SW620, and SW837). Many markers that were not seen in more than one study, though clonal, were only present in a few cells and perhaps represent novel or unstable rearrangements. LS411N has been reported by only one other group (Abdel-Rahman et al., 2001), and only about one-fourth of the abnormalities were in common with our data. The 10 aneuploid cell lines exhibited complex karyotypes with many numerical and structural abnormalities. COLO320DM was the most complex of the cell lines studied (Figs. 1A–1D), exhibiting four subclones and 45 chromosomal abnormalities, many of which were complex rearrangements, including hsr's and dmin's; only eight of the abnormalities had been previously reported (Tsushimi et al., 2001; Kleivi et al., 2004).

Although the two lines originating from the same patient, SW480 (from a primary tumor) and SW620 (from a metastatic lymph node obtained the following year), were cytogenetically quite dissimilar, they shared two common abnormalities [the t(5;20)/der(20)t(5;20) markers and +11], and Melcher et al. (2000) noted that the two lines shared common gains (5q15→5q11, 7pter→q22, +11, 13q14→qter, 20pter→p13, +X), common losses (8pter→8p2, 18q12→qter, −Y), and common breakpoints (5q11, 5q15, 7q22, 13q14, 18q12, 20p12), supporting the conclusion that the two lines shared a common founder cell. Each line was quite stable when compared with previous studies: the majority (18 of 24) of the clonal abnormalities found in SW480 had been previously reported (Melcher et al., 2000; Abdel-Rahman et al., 2001; Camps et al., 2004a,b; Kleivi et al., 2004), and two-third of the abnormalities observed in SW620 were also seen in other studies (Melcher et al., 2000; Abdel-Rahman et al., 2001; Roschke et al., 2003). Despite being derived from a metastatic tumor, SW620 demonstrated fewer (20 vs. 24) cytogenetic abnormalities than SW480 and a lower modal number of chromosomes (50 vs. 58). Our CGH results for SW480 were similar to those reported by Abdel-Rahman et al. (2001); they found fewer CGH gains and losses in SW620 than in SW480 (involving 13 vs. 20 chromosomes, respectively).

p53HCT116 is a *TP53*-mutant line derived from HCT116 (Bunz et al., 1998). The two lines share several structural abnormalities except that the mutant line contains a translocation t(5;7)(q13;p22). Both lines contained a der(18)t(17;18)(q21.3;p11.3): p53HCT116 had no involvement of chromosome 4, while HCT116 had der(4)t(4;17)(q3?;?q21) and two variants of the der(18): the der(18)t(17;18) plus a complex rearrangement involving chromosome 4: der(18)(:4q3?::17q?22→17q21.3::18pter→18qter); the SKY results were confirmed by FISH. Chromosomal CGH showed gains of 8q and 17q but a balanced state for chromosome 4 (Fig. 2); however, array CGH studies detected a subtle deletion of chromosome 4 mapping to the (4;17) junction in both cell lines (Camps et al., 2009).

With respect to ploidy levels, 8 of the 10 aneuploid lines had hyperdiploid or triploid karyotypes, as reported in previous studies. Numerical abnormalities were much less prevalent than were structural ones; the only recurrent numerical abnormalities detected were loss of the Y chromosome in 9 of the 12 male cell lines; loss of chromosomes 13, 14, 15, 18, 19, and 21 in three to four cell lines each; gain of chromosome 11 in five lines; and gain of 7, 12, 13, and 20 in three to four cell lines each.

SKY analysis revealed 407 chromosomal breakpoints in the CRC cell lines (Fig. 3); each particular breakpoint was counted only once per cell line, even if it was involved in several abnormalities in that cell line. A total of 259 breakpoints were recurrent, i.e., seen in more than one cell line (Table 2 and Fig. 3): 88 of the recurrent breakpoints (34%) were located in the centromeric (p11→q11) or pericentromeric (p11.2 and q11.2) regions and 34 (13%) recurrent breakpoints were in the terminal bands of 14 different chromosomes. The most common centromeric/pericentromeric breakpoints involved chromosomes 12, 13, and 20 (eight to nine cell lines each), followed by chromosomes 14, 15, 17, and 18 (five to

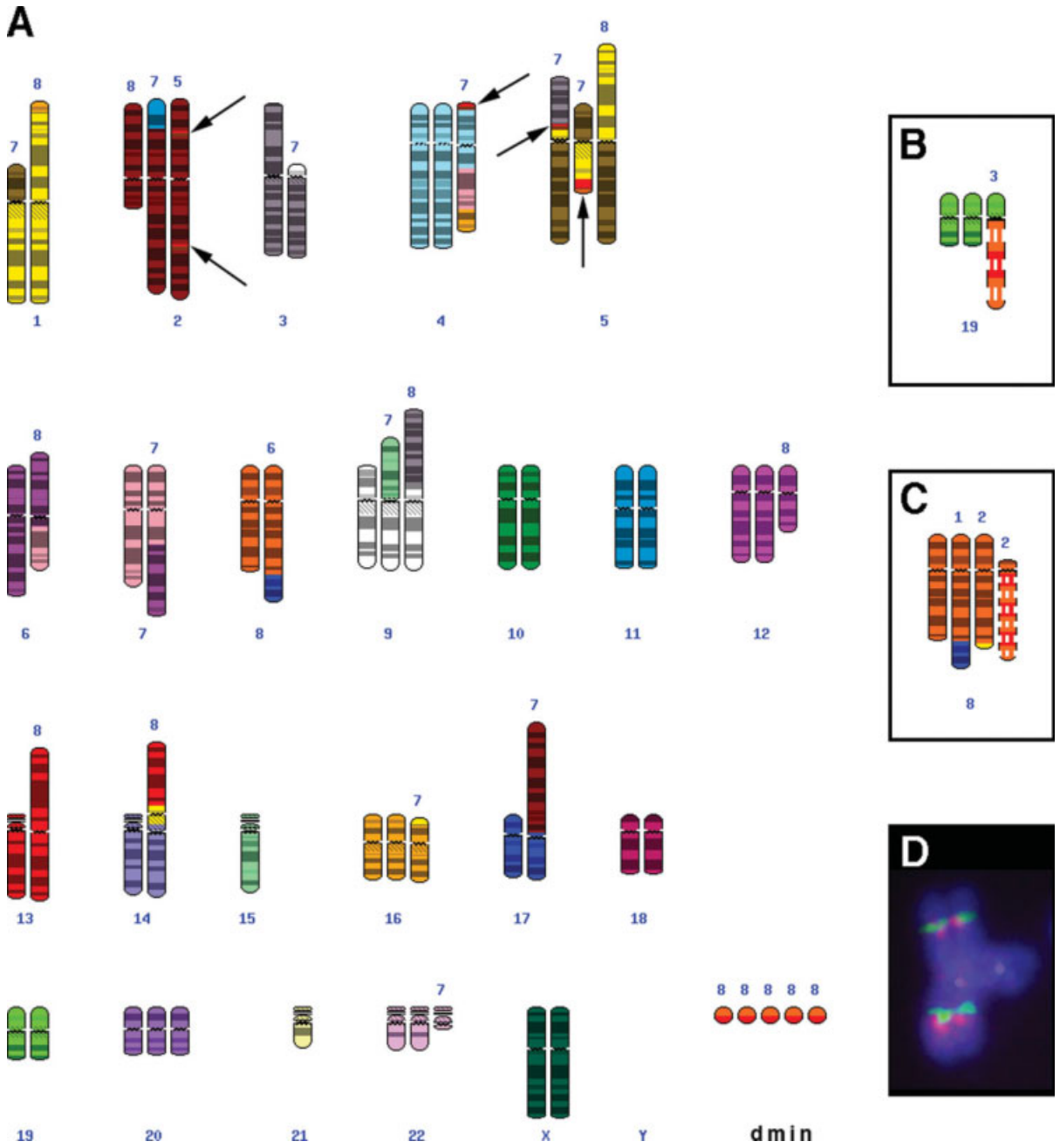

Figure 1. SKYGRAM ideograms in COLO320DM demonstrating complex chromosomal aberrations, including involvement of chromosomes 8 (orange, *MYC*+) and 13 (red, *CDX2*+): (A) double minutes and translocations/insertions (arrows) in clone A1. (B) hrs in der(19) in clone B1. (C) hrs in der(8) in clone B2. Clones A1 and A2 contained 50–100 dmin per cell. See Table 1 for full written karyotypes. (D) FISH in COLO320DM: insertions of *MYC* (orange) and *CDX2* (green) into chromosome 2.

seven cell lines). Each recurrent terminal band breakpoint was present in two to four cell lines (Table 2), the most common being 8q24 and 11p15.

The specific types of structural abnormalities found in each cell line are listed in Table 3. All chromosomes displayed structural abnormalities. There were 150 clonal translocations; only eight were balanced (three occurred in COLO320DM and the remaining were seen in one cell line each). The diploid lines had 1–5 translocations per cell line (mean 2.4, median 1) and the aneuploid lines had 4–29 translocations per line (mean 13.8, median 12.5). Unbalanced translocations were by far the most frequent structural abnormalities. The diploid lines had few other types of

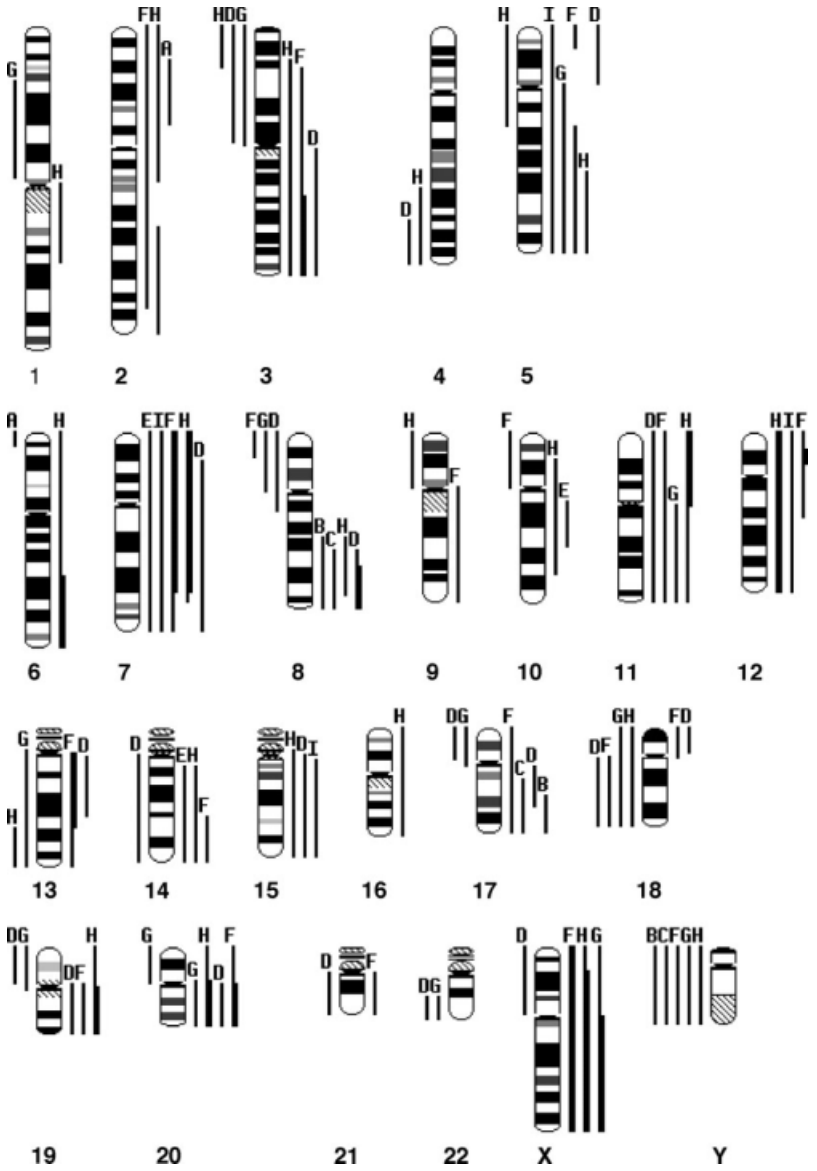

Figure 2. Chromosomal CGH in nine CRC cell lines: A, DLD1; B, HCT116; C, p53HCT116; D, HT-29; E, SW48; F, SW480; G, SW837; H, T84; and I, LoVo. Vertical lines to the left of the chromosome ideogram indicate loss and lines to the right indicate gain; heavy lines indicate amplification.

structural abnormalities and no hsr's or dmin's, whereas the aneuploid lines displayed numerous deletions, duplications, isochromosomes, hsr's, and dmin's. Of the 22 amplicons identified by aCGH in the aneuploid lines, nine were located near sites of chromosomal translocations involving chromosomes 1, 2, 8, 12, 20, and the X; five were present as hsr's; and six were present as dmin's in three cell lines. The mechanism of amplification was not determined for three of the amplicons. Some amplicons displayed more than one type of mechanism (e.g., dmin and hsr). *MYC*, located at 8q24.21, was involved in hsr's, dmin's, and jumping translocations (JTs) in combination with band 13q12 in COLO320DM as revealed by SKY and FISH analysis (Figs. 1A–1D). *MYC* amplification was observed in this cell line and in three other cell lines: NCI-H716 (as dmin), SW480 (as the result of a translocation, Figs. 4A and 4B), and HT-29 (as an hsr). Three amplicon-containing cell lines were studied by chromosomal CGH: three of their five amplicons showed amplification with this technique. A thorough investigation of the genes within the amplicons,

Figure 3. Recurrent breakpoints in 15 CRC cell lines. Each mark indicates one cell line (each particular breakpoint was counted only once per cell line). Color code: blue, translocation; red, deletion; pink, interstitial deletion; brown, duplication; plum, insertion; green, isochromosome; orange, hsr; light blue, multiple types of breakpoint per band in one cell line (e.g., translocation and deletion).

TABLE 2. Recurrent Chromosomal Breakpoints in Colorectal Cancer Cell Lines

Chromosome band	No. of cell lines	Chromosome band	No. of cell lines	Chromosome band	No. of cell lines
Xp22.1	2	6q27[a]	2	**13p11.1**	**2**
Xp21	2	7p22[a]	2	**13q10**	**6**
Xq28[a]	2	7p15	3	**13q11**	**2**
1p12	3	7p13	2	13q12	3
1q10	**3**	**7p10**	**2**	13q14	4
1q12	2	**7q11.2**	**2**	13q22	2
2p22	2	7q21	2	**14q10**	**3**
2p21	2	7q22	6	**14q11.2**	**4**
2q21	4	7q36[a]	3	14q12	2
2q22	2	8p23[a]	2	14q13	2
2q24	2	8p12	2	14q22	2
2q31	2	**8q10**	**2**	14q24	3
3p25	3	**8q11.2**	**2**	**15q10**	**5**
3p22	3	8q13	3	15q22	2
3p21	4	8q22	3	16p13.3[a]	2
3p12	2	8q24.1-q24.3[a]	4	16q23	2
3q10	**3**	9p13	4	**17q11.2**	**3**
3q21	2	**9q11**	**4**	**17q11.2**	**3**
4p16[a]	2	9q34[a]	2	17q21	4
4q31.1-q31.3	3	**10p11.2**	**2**	17q25[a]	2
5p13	2	10q21	2	**18p11.2**	**4**
5p12	2	10q23	4	**18p10**	**2**
5p10	**4**	10q26[a]	2	18q21	2
5q13	2	11p15[a]	4	19q12	2
5q14.2	2	11p14	2	19q13.1	2
5q15	2	**11p11.2**	**2**	20p12	2
5q33	2	11q13	2	**20p11.2**	**3**
6p25[a]	3	11q21	2	**20q10**	**4**
6p23	2	12p12	2	**20q11.2**	**2**
6p10	**2**	**12p11.2**	**3**	20q13.3[a]	2
6q14	2	**12p10**	**3**	**21q10**	**2**
6q22	2	**12q11**	**3**	**22p11.2**	**2**
6q25	2	12q13	2	**22q11.2**	**2**
6q23	2	**13p11.2**	**2**		

Boldface indicates centromeric and pericentromeric regions.
[a]Terminal band.

as well as expression levels of these same genes in cell lines and tumors without amplicons, is presented by Camps et al. (2009).

Of the 150 clonal translocations exhibited in these lines, 21 (14%) were JTs (Table 4, Figs. 4C and 4D). JTs are unbalanced translocations involving a donor chromosome arm or chromosome segment fused to multiple recipient chromosomes within one sample (Lejeune et al., 1979); the donor segments have the same breakpoints. Eight lines had JTs: four lines had only one JT, while four lines had 3–5 JTs each. JTs were most frequent in the cytogenetically complex cell lines and only one JT was seen in a diploid line (HCT116). The 21 JTs involved 45 different breakpoints, donor arms or segments from 13 different chromosomes, and 17 different recipient chromosomes. The number of different donors in each cell line ranged from one to five and the number of recipients was generally two–three per donor; the highest number of recipients was observed in COLO320DM, which displayed insertion of material containing *MYC/CDX2* (8q24.21/13q12) into 10 different chromosomal locations; this cell line also had hsr's and dmin's composed of both of these genes. The majority of JTs (14/21) involved chromosome segments rather than whole arm translocations. The donors in the eight cell lines with JTs exhibited no recurrent fusion breakpoints with respect to recipient chromosomes; only chromosome 8 was involved more than a few times, each time with a unique breakpoint (Table 4). There was also no consistency among the recipient chromosomes.

The only recurrent structural abnormalities in these cell lines were all isochromosomes (Table 4), which were observed in 1 of the 5 diploid and 9 of the 10 aneuploid lines; the total number of

TABLE 3. Number of Clonal Abnormalities per CRC Cell Line

Cell line	Ploidy	Tot Abn[a]	Num (+/−)	Tot Trans	JT	Iso Chr	Dup[b]	Ins	Inv	Del	hsr[b]	dmin[b]	Misc
DLD1	Diploid	2	0	1	0	0	1(2)	0	0	0	0	0	0
HCT116	Near-diploid	7	1	5	1	0	1(10)	0	0	1	0	0	0
P53HCT116	Near-diploid	5	1	4	0	0	1(10)	0	0	0	0	0	0
SW48	Near-diploid	5	3	1	0	0	1(10)	0	0	0	0	0	0
LoVo	Near-diploid	5	3	1	0	1	0	0	0	0	0	0	0
Subtotal	–	*24*	*8*	*12*	*1*	*1*	*4*	*0*	*0*	*1*	*0*	*0*	*0*
HT-29	Triploid	26	6	8	0	7	1(19)	1	0	5	1(8)	0	0
SW480	Hyperdiploid	24	6	15	1	2	1(3)	0	1	0	0	0	0
SW620	Hyperdiploid	20	6	9	3	0	0	0	0	5	1(16)	0	0
SW837	Hypodiploid	17	5	9	1	1	0	0	0	2	0	0	0
LS411N	Triploid	25	3	13	5	6	0	0	0	2	1(7)	0	1 ace (18)
COLO320DM	Hyperdiploid	45	9	29	4	2	1(6)	5	0	4	1(8/13)	1(8,13)	0
T84	Hyperdiploid	30	6	16	0	2	2(6,11)	0	0	5	0	0	0
NCI-H508	Tetraploid	21	12	4	0	2	0	0	0	3	0	1(14)	0
NCI-H716	Triploid	37	19	12	1	2	0	0	0	3	0	2(8,10)	0
SK-CO-1	Triploid	54	4	23	5	3	2(11,12)	0	0	20	4(6,18,20,22)	0	1 min (17)
Subtotal	–	*299*	*76*	*138*	*20*	*27*	*5*[c]	*6*	*1*	*49*	*5*[c]	*3*[c]	*2*
Total	–	*323*	*84*	*150*	*21*	*28*	*9*[c]	*6*	*1*	*50*	*5*[c]	*3*[c]	*2*

Tot Abn, total abnormalities; Num, numerical abnormalities; Tot Trans, total translocations, JT, jumping translocation, Iso Chr, isochromosome; Dup, duplication; Ins, insertion; Inv, inversion, Del, deletion; hsr, homogenously staining regions; dmin, double minute; ace, acentric fragment; min, minute chromosome.

[a]Total number abnormal chromosomes: abnormalities are not included in the total number of abnormalities if they are part of another abnormality (e.g., dup, ins, and hsr in HT-29 were all part of complex abnormalities).

[b]Chromosomes involved in dup, hsr, or dmin in parentheses.

[c]Number of cell lines involved.

isochromosomes was 27 and the number per cell line ranged from one to seven. HT-29 had the most isochromosomes (seven), followed by LS411N (six), and SK-CO-1 (three). Isochromosomes involving 13q and 14q were observed in three cell lines; 8q, 12p, 18p, 20q, and 21q in two cell lines each; and the remaining in one cell line each. In some cases, the isochromosomes also contained duplications and deletions of chromosome arms [e.g., ider(13)(q10)del(13)(q14) and ider(19)(q10)dup(19)(q13.1q13.4) in HT-29].

Cancer Chromosomes Database

The Similarity Report tool in the Cancer Chromosomes database was used to analyze breakpoints in the 345 cases of adenocarcinoma of the large intestine in the Mitelman database (Mitelman et al., 2008). Breakpoints in the tumors were predominantly located in the centromeric and terminal band regions (Table 5): of the 50 most common recurrent breakpoints, 50% (296 of a total of 591 breakpoints) were located in the centromeric regions and 11% (64) were in terminal bands. In descending order of frequency, the most common breakpoints in centromeric regions were those of chromosomes 17 (16% of 591 breakpoints), 8 (12%), 1 (7%), 13 (6%), 5 (4%), and 7 (4%).

In comparing the recurrent cytogenetic abnormalities in the 345 cases in the Mitelman database (Table 6), we found that the results of the cell line studies mirrored those reported in primary tumors: balanced rearrangements were rare (only one reported) and the most common structural abnormalities were isochromosomes, the most frequent (in descending order) involving chromosome arms 8q, 17q, 13q, 1q, 5p, and 7p. Examination of junctions (Table 7) revealed that isochromosomes are also the most common type of structural abnormality in CRC tumors, and that no other specific junctions were observed in more than six cases each. The recurrent abnormalities tool of the Mitelman Database revealed that, after isochromosomes, the most frequent abnormalities were deletions; no specific deletion was seen in more than nine cases (<3%) (Table 6). Deletions were infrequent in the cell lines (Table 3).

CGH

The CGH profiles of 9 of the 15 cell lines are shown in Figure 2. The most common gains (in four or more cell lines) were in chromosomes or

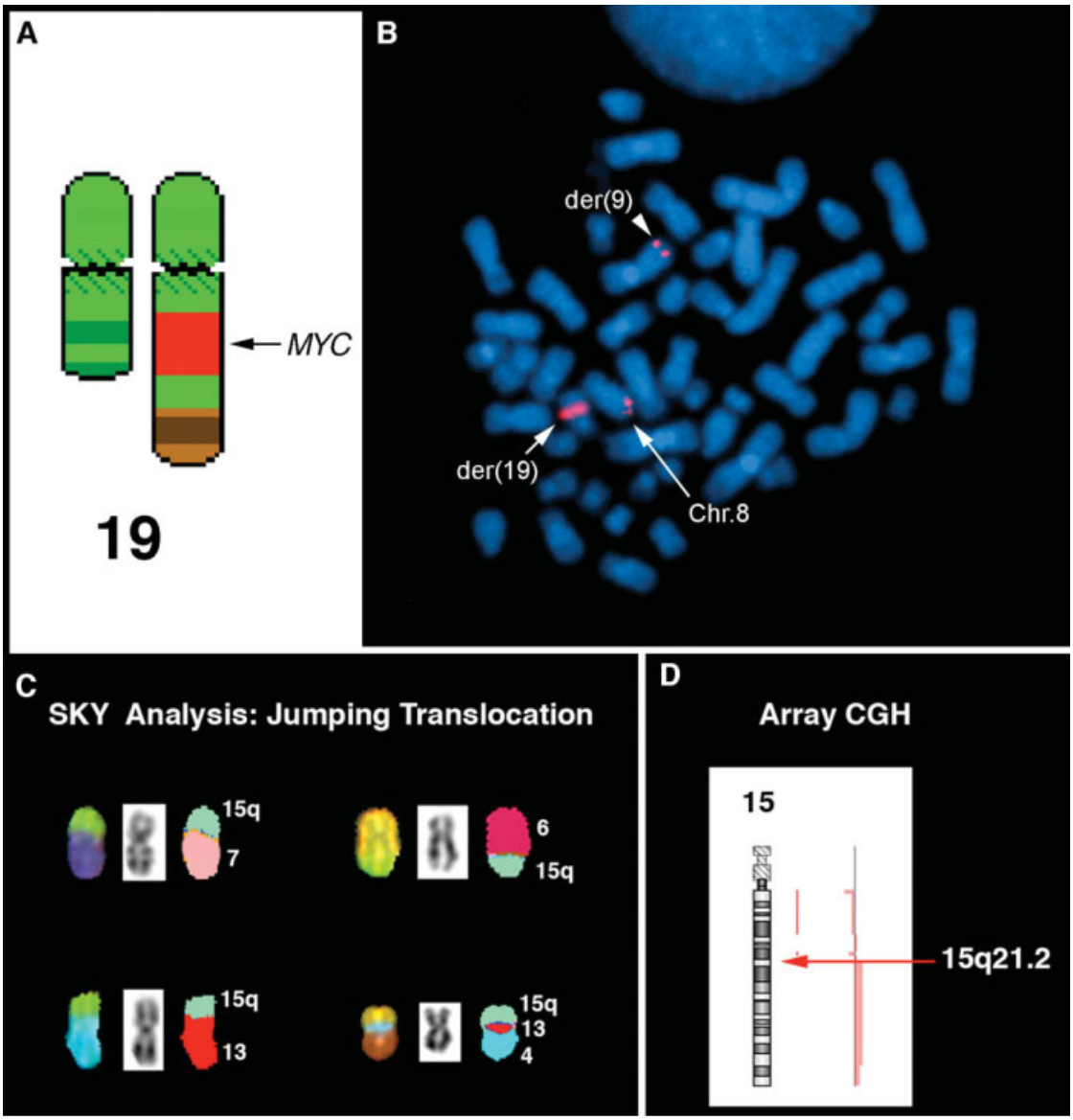

Figure 4. Cell line SW480. (A) Partial SKYGRAM demonstrating der(19)t(8;19)t(5;19); the arrow indicates chromosome 8. (B) FISH demonstrating copies of *MYC* in a normal chromosome 8 and a der(9), and *MYC* amplification in the der(19). (C, D) Jumping translocations involving chromosome 15 in LS411N: (C) SKY analysis and (D) aCGH demonstrating amplification of the JT segment 15q21.2→15qter.

chromosome arms 3 and 3q, 5q, 7, 8q, 1, 12, 20, 20q, and X (in descending order of frequency). The most common losses (three or more lines) were in chromosomes/chromosome arms 8p, 18, and Y. As expected, the five diploid lines showed far fewer gains and losses than did the four aneuploid lines. In all nine cell lines, the CGH results matched well with the results of the SKY studies. Gain of 13, a common feature of CRC, was only observed in two lines; this finding is most likely due to the fact that only four of our lines studies by chromosomal CGH were of the aneuploid type.

DISCUSSION

We have presented a cytogenetic study of 15 CRC cell lines using SKY, FISH, and CGH; molecular cytogenetic studies on five of these lines are presented for the first time. The 10 aneuploid cell lines with chromosome instability exhibited complex karyotypes and many more

TABLE 4. Isochromosomes and Jumping Translocations in 15 Colorectal Cell Lines

		Jumping translocations (JT)[a]				
Cell line	Isochrom.	Donor arm/segment	Donor fusion breakpoint	No. of JT	Recipient fusion breakpoint	Gain aCGH
DLD1	None	None				
HCT116	None	17q21-q22→17qter	17q21	2	4q3?, 18pter	Yes
p53HCT116	None	None				
SW48	None	None				
LoVo	15q	None				
HT-29	3q, 8q[b], 13q[b], 18p, 19q[b], 20q	None				
SW480	7q, 12p	14q22→14qter	14q22	2	7q22, 9q34	Yes
SW620	None	8p23→8qter	8p23	2	17q25, 13q14.3	ND
		10pter→10q23	10q23	3	14q24, 13q14.3, hsr16	
		13q14.3→13qter	13q14.3	2	8p23, 10q23	
SW837	20q	Xpter→Xq28	Xq28	2	5q13, 8q22	No[c]
LS411N	5p, 6p, 6q, 12p, 14q, 21q	8q	8p11.2/8q10	2	9p10, 12q21	No
		7p13→7qter	7p13	2	1p12, 8q11.1, 15q21.2	Yes
		12q21.2→12qter	12q21.2	2	7hsr, 8p11.2	Yes
		Xp22.1→Xqter	Xp22.1	2	5q13, 14q11	Yes
		15q21.2→15qter	15q21.2	4	6q23, 7p13, 13q?12, 13q22	Yes
COLO320DM	13q[b], 14q	7q22.3→7q36	7q22.3	2	4q21.21, 6q12	Yes
		MYC + *CDX2*	8q24.21+13q12	10	1q23.1, 2p21, 2q31,3q22.1, 4p16.3, 4q21.21, 8q11.2, 10q24, 15q22, 19q13.4	Yes
		5p12→5qter	5p12	2	1p12, 1p11.2	No
		14q11.2→14qter	14q11.2	3	4q21.21, 7p22, 8q24.21	Yes
	T84	Xq, 13q	None			
NCI-H508	1q[b], 17q	None				
NCI-H716	10p, 21q	20q	20q11.2	3	Xq11.2, 13q14.1, 22p11.2	Yes
SK-CO-1	8q, 14q, 18p	1q	1q11	2	5p11, 9p11	Yes
		8q22.3∼23→8qter	8q22.3∼23	2	14q22, 17q25	Yes
		13q	13p11.2	2	10p11.2, 14q11	Yes
		17q11.2→17q25	17q11.2	2	1q11, 2q11.1	No
		11q12.2→11q14	11q12.2	3	9q12, 7q, 18hsr	Yes

[a]NB: Some JTs involved nonclonal rearrangements.
[b]Isochromosomes: HT-29: der(8)i(8)(q)hsr; ider(13)(q10)del(13)(q14); ider(19)(q10)dup(19)(q13.1q13.4); COLO320DM: der(13;13) (qter→q21::q21→q10::q10→qter); NCI-H508: idel(1)(q25.1→q10::q10→q25.1).
[c]SW837: Possible dup(8)(q28) confirmed by aCGH.

TABLE 5. Fifty Most Common Breakpoints in 345 Cases of Adenocarcinoma of Large Intestine Listed in the Mitelman Catalog

Breakpt.	No. of cases	Breakpt.	No. of cases	Breakpt.	No. of cases	Breakpt.	No. of cases
1p36[a]	8	**5p10**	**16**	**8q11**	**10**	16p13[a]	9
1p34	7	**5q11**	**7**	9p21	7	16p12	7
1p32	8	5q21	7	9q22	8	17p	8
1p22	13	5q31	8	9q34	7	17p12	16
1p13	12	6q13	8	10q24	7	**17p11**	**34**
1p11	**13**	6q15	7	11q13	8	**17q10**	**47**
1q10	**17**	6q22	7	11q23	12	**17q11**	**11**
1q11	**10**	7p15	12	12q13	7	18q21	15
1q21	8	**7p10**	**12**	12q24[a]	9	19p13[a]	10
2q37[a]	8	**7q11**	**9**	**13p11**	**7**	19q13[a]	7
3p21	9	7q32	9	**13q10**	**29**	20p13[a]	13
3q13	7	**8p11**	**11**	**14q10**	**8**		
3q21	7	**8q10**	**48**	**15q10**	**7**		

Boldface indicates centromere region.
[a]Terminal band.

TABLE 6. Recurrent Structural Abnormalities in 345 Cases of Adenocarcinoma of the Large Intestine from the Mitelman Catalog[a]

Structural abn.	No. of cases	Structural abn.	No. of cases
Balanced		**Unbalanced**	
inv(16)(p13q22)	2	del(8)(p21)	3
		der(8;17)(q10;q10)	4
Unbalanced		**i(8)(q10)**	**42**
add(1)(p11)	6	del(9)(p21)	7
del(1)(p11)	3	del(9)(q22)	5
del(1)(p13)	6	del(10)(q22)	4
del(1)(p22)	9	del(10)(q24)	5
del(1)(p32)	5	add(11)(q23)	3
del(1)(p34)	6	del(11)(q23)	6
del(1)(p36)	4	i(12)(p10)	5
i(1)(q10)	**13**	del(12)(p12)	4
del(1)(q11)	6	add(12)(q24)[b]	4
del(1)(q32)	3	add(13)(p11)	3
del(2)(p23)	4	**i(13)(q10)**	**21**
i(4)(q10)	3	i(14)(q10)	4
i(5)(p10)	**13**	del(16)(p12)	6
del(5)(q14q23)	3	add(16)(p13)[b]	6
del(5)(q15q31)	4	**add(17)(p11)**	**11**
del(5)(q21q31)	3	**del(17)(p11)**	**11**
i(6)(p10)	6	**del(17)(p12)**	**12**
del(6)(q13)	7	der(13;17)(q10;q10)	3
del(6)(q15)	4	der(8;17)(q10;q10)	4
del(6)(q22)	3	**i(17)(q10)**	**36**
del(6)(q23)	3	**del(18)(q21)**	**13**
i(7)(p10)	**12**	add(19)(p13)[b]	4
del(7)(p15)	5	del(19)(p13)	3
add(7)(q11)	3	add(19)(q13)[b]	5
del(7)(q32)	3	add(20)(p13)[b]	5
del(8)(p11)	3	i(20)(q10)	3

Boldface indicates most frequent abnormalities.
[a]Accessed January 2008.
[b]Terminal bands.

TABLE 7. Chromosome Junctions in 345 Cases of Adenocarcinoma of the Large Intestine from the Mitelman Database[a]

Junction	No. of cases	Isochrom.	Junction	No. of cases	Isochrom.
1q10 1q10	11	x	13q 17p	6	
2p13 2q37	2		13q10 1q10	2	
2q10 2q10	3	x	13q10 13q10	21	x
3p21 20p13	2		14q10 14q10	3	x
4q10 4q10	3	x	15q10 15q10	2	x
4q35 3p21	2		16p13 16q22	2	
5p10 5p10	13	x	16q13 15q11	2	
5q12 5q22	2		17p11 20p12	2	
5q14 5q23	3		17q10 8q10	4	
5q15 5q31	4		17q10 17q10	34	x
5q21 5q31	3		17q10 13q10	3	
6p10 6p10	6	x	19p11 8q11	2	
7p10 7p10	12	x	20p13 8q11	2	
7q10 7q10	2	x	20p12 17q21	2	
8p12 15q11	2		20p11 20p11	2	x
8q10 8q10	42	x	20q10 20q10	2	x
9p12 9q13	2		21p13 21p13	2	
9q10 9q10	2	x	22q10 13q10	2	
11q13 17q21	2		Xq10 Xq10	2	x
12p10 12p10	4	x	Single Junct.	6	

[a]Data extracted using the "similarities" tool in the NCI/NCBI Cancer Chromosomes database, which includes the Mitelman Database of Chromosome Aberrations in Cancer, accessed January 2008.

abnormalities than did the five diploid lines characterized by defects in DNA mismatch repair (MMR^-). A strong correlation was seen between the chromosomal aberrations identified in the SKY analysis and the genomic imbalances revealed by array CGH in which 87% of the rearrangements observed by SKY correlated with the presence of a gain or loss at the same breakpoint. We also reviewed the karyotypes of 345 cases of carcinoma of the large intestine listed in the Mitelman Database of Chromosome Aberrations in Cancer and compared those results with the ones observed in the cell lines. The types of abnormalities seen in the cell lines reflected those seen in direct tumors and can be considered characteristic of CRC: there were no recurrent translocations and only a few balanced translocations, isochromosomes were the only recurrent abnormalities (small numbers of recurrent deletions were noted only in the primary tumors), and breakpoints were most frequent at centromeric/pericentromeric regions, which can lead to isochromosome formation.

This is similar to another comprehensive study of the genomic profiles of 20 colon cancer cell lines by Kleivi et al. (2004), which included five of the lines in this study (HCT116, LoVo, SW48, COLO320, and SW480). As in this study, they found that the near-diploid lines demonstrating microsatellite instability (MMR^-) showed considerably fewer aberrations (mean 2.6) than did aneuploid lines exhibiting chromosome instability (MMR^+) (mean 8.5 copy number changes). In comparing their G-banding karyotypes, CGH, and M-FISH analyses with previous studies in the literature, they observed more differences of numerical aberrations than structural abnormalities. They also found no recurrent translocations, which they wrote supported "... the notion that fusion protein and overexpression of oncoproteins caused by such aberrations do not play an important role in colorectal tumorigenesis." The results of a companion study examining the gene expression profiles in these cell lines support their conclusion (Camps et al., 2009). Translocations were not found to preferentially affect genes within close proximity to the breakpoints, rather they served instead as boundaries

for copy number changes, affecting the average gene expression along the length of the entire affected chromosome segment.

The CGH profiles for the nine cell lines in this study matched very well with those results described in earlier studies (Abdel-Rahman et al., 2001; Kleivi et al., 2004). Abdel-Rahman et al. (2001) reported the most frequent gains in 1q, 2, 3q, 5, 7, 8q, 9, 10, 11, 12, 17q, and 20 and the most common losses in 8p and 18q; they determined that the patterns of genomic change shown by CGH reflected those of primary tumors reported in the literature. Kleivi et al. (2004) observed few copy number changes in the diploid lines; the most frequent gains in the aneuploid lines involved 20, 11, and 8q, and the most frequent losses at 18q (100%), X, 4q, and 17p. Amplifications at 8q23∼24, 12p, and Xq28 were present in at least two of the cell lines they analyzed, consistent with our observation of amplification at all three sites by aCGH. Of the three cell lines we studied by CGH in common with Kleivi et al. (2004), amplification of 8q was found in COLO320DM and HT-29, and chromosome 7 in SW480.

CGH analysis of primary colon cancer tumors have been reported by a number of investigators (Ried et al., 1996; Camps et al., 2008), the Progenetix database (Baudis, 2008), and in Diep et al.'s unpublished literature survey of CGH in 669 primary CRC cases cited in Kleivi et al. (2004). In summarizing the CGH patterns in all of these reports, gains were most frequent for 3/3q, 5/5p/5q, 7, 8q, 20/20q, 13, and the X, and losses were most frequent for 8p and 18/18q. This overall pattern matches quite well with the CRC cell lines in our study.

With regard to a comparison of the cell lines representing a primary and metastatic tumor derived from the same patient (SW480 and SW620, respectively), two other groups have also compared the SKY karyotypes of both lines (Melcher et al., 2000; Abdel-Rahman et al., 2001). The results were very similar in these two studies, and although the cytogenetic abnormalities were quite different between the two lines, a few specific common abnormalities, as well as several common breakpoints and areas of gains and losses as noted by Melcher et al. (2000), confirmed that they shared a founder cell. These results are consistent with the model of Klein (2009) in which metastasis occurs as an early event in tumor progression, hence the common aberrations, and that further independent evolution at the primary and secondary sites would result in divergent karyotypes. Another view, put forth by Dutrillaux (1995), states that aneuploid colon cancers (and breast cancers as well) evolve by losing chromosomes since unbalanced translocations often result in net chromosome loss, which can be followed by endoreduplication to produce near-triploid cells with duplicated abnormalities. In this model, the metastatic potential of tumor cells would be acquired as a later event through continued loss of genomic material. Our SKY studies demonstrating that SW480 and SW620 had modal numbers of 58 and 50 chromosomes, respectively, as well as the CGH analysis of Abdel-Rahman et al. (2001) in which SW620 displayed fewer CGH gains and losses than did SW480 are consistent with this model. Further support comes from an earlier study by Gagos et al. (1996) in which SW480 and SW620 were continuously cultured for 2 years, with periodic chromosome banding analysis. They noted that genomic instability, including telomeric association and random dicentric and multicentric formation, led to the disappearance of particular sidelines through evolution. The instability was characterized by the elimination of pre-existing marker chromosomes and subsequent replacement with their intact homologous chromosomes, possibly after selective endoreduplication, thereby leading to loss of heterozygosity. One example involved the SW480 markers t(1;9)(q12;q11) and der(2;12)(q22;q12). Both markers were present in early passages of SW480 (and in our SKY analysis), but were absent in the later passages of SW480 as well as the metastatic line SW620. The authors suggested that continuous clonal diversification may be a way for cancer cells to bypass senescence and that these rearrangements may be the result of combined action of telomeric loss and restoration, and nondisjunction.

The absence of telomerase activity in most cell types results in the erosion of chromosome ends with each subsequent division, ultimately leading to senescence as cells reach the Hayflick limit with dangerously short telomeres. Bypassing this cellular blockade and becoming immortalized is a critical step in tumorigenesis and often involves reactivation of telomerase activity (Maser and DePinho, 2002). At some point, however, the shortened telomeres may become lost and/or recombinogenic. Our analysis uncovered the presence of 36 rearrangement events involving a telomeric chromosome band (Table 8). Further

TABLE 8. Terminal Break Mechanisms

Cell line	Terminal band breaks	Mechanism
HT29	3q	Telomere capture
HT29	Xq	Telomere capture
SW480	9q	Telomere capture
SW480	8p	Translocation
SW837	11p	Telomere capture
SW837	Xq	Telomere capture
SW837	Xq	Telomere capture
SW837	7q	Translocation
T84	2p	Translocation
T84	7q	Telomere capture
T84	11p	Translocation
T84	6q	Telomere capture
T84	20q	Telomere capture
LS411N	None	None
NCI-H508	6q	Telomere capture
NCI-H508	6q	Telomere capture
NCI-H716	2q	Telomere capture
LoVo	None	None
SW48	22q	Telomere capture
HCT116	4q	Telomere capture
HCT116	16p	Telomere capture
HCT116	18p	Telomere capture
HCT116	10q	Telomere capture
p53HCT116	16p	Telomere capture
p53HCT116	18p	Telomere capture
p53HCT116	10q	Telomere capture
p53HCT116	7p	Translocation
DLD1	6p	Telomere capture
Colo320DM	4p	Translocation
Colo320DM	8q	Telomere capture
Colo320DM	8q	Undetermined
Colo320DM	7p	Undetermined
SK-CO-1	4p	Telomere capture
SK-CO-1	11p	Undetermined
SK-CO-1	18p	Undetermined
SK-CO-1	20p	Translocation
SW620	8p	Telomere capture
SW620	8p	Telomere capture

investigation revealed that 70% of these chromosomes were unbalanced with respect to the fusion partner, as evidenced by the presence of the modal number of intact copies of the homologous chromosome and by aCGH. While the clonal evolution described earlier by Gagos et al. (1996), involving loss of marker chromosomes and subsequent endoreduplication of the normal homologue, would be consistent with these observations, another mechanism described for the generation of unbalanced translocations is break-induced replication (Morrow et al., 1997; Bosco and Haber, 1998; Signon et al., 2001). We have previously observed this phenomenon in animal models of pro-B cell lymphomas where a portion of the IgH locus on mouse chromosome 12 is copied next to *Myc* on a broken chromosome 15, resulting in a gain of that region of the IgH locus containing the enhancer (Difilippantonio et al., 2002). Failure to copy a segment containing a telomere sequence results in an unstable, recombinogenic chromosome that is prone to repeated rounds of the breakage-fusion-bridge cycle (Schimke, 1982; Smith et al., 1995; Coquelle et al., 1997) until the eventual "capture" of a telomere (Meltzer et al., 1993), often again through break-induced replication (Difilippantonio et al., 2002).

Isochromosomes are the most frequent recurrent aberrations in epithelial cancer (Mertens et al., 1994). They are present in almost 10% of all neoplasms with cytogenetic aberrations and are relatively more frequent in solid tumors. Isochromosome formation often leads to gain or loss of genetic material and does not appear to lead to structural rearrangements of cancer-related genes. They are seldom seen as sole abnormalities and are generally interpreted as secondary changes related to tumor progression. In the 345 cases of adenocarcinoma of the large intestine reported in the Mitelman Database (Mitelman et al., 2008), isochromosomes were by far the most common recurrent abnormalities and the most frequent involved chromosome arms 8q (42 cases), 17q (34), 13q (21), 1q (13), 5p (13), and 7p (12) (Table 6). Consistent with this, all of these isochromosomes were found in our cell lines with the exception of 7p. The mechanism creating isochromosomes is unknown, although there is support for two hypotheses: transverse rather than the normal longitudinal division of the centromere or translocation and chromatid exchange involving two homologous chromosomes (Mertens et al., 1994). Since only 1 of 28 isochromosomes found in this study occurred in the diploid lines, the results by Ghadimi et al. (2000) demonstrating the presence of centrosome amplification and instability solely in the aneuploid CRC cell lines supports the notion of segregation errors in isochromosome formation. It should also be noted that the only diploid line (LoVo) displaying an isochromosome, while being MMR deficient, was found by Ghadimi et al. (2000) to have intermediate impairment of centrosome function.

Eight of the cell lines exhibited JTs (Table 4). JTs are constitutional or acquired chromosomal rearrangements involving one donor and several recipient chromosomes within the same sample (Lejeune et al., 1976). Among malignancies, they have been reported most frequently in

hematological conditions (Sawyer et al., 2005) and there have been few reports of JT in solid tumors (Padilla-Nash et al., 2001; Roschke et al., 2003). Padilla-Nash et al. (2001) reported many JTs and SJTs (segmental JT) in a variety of solid tumor cell lines (none of which were derived from CRC). They noted that the breakpoints also coincided with centromeric and pericentromeric regions or regions of chromosome instability such as fragile sites and viral integration sites, and that JTs resulted in copy number gains of the donor segments that often contained oncogenes such as *MYC*; in some instances, JTs contributed to clonal progression. The precise mechanism of JT formation is unknown; however, Sawyer et al. (2005) proposed that hypomethylation of DNA in pericentric regions results in decondensation of donor pericentric heterochromatin that permits fusion of this segment to other pericentromeric or telomeric heterochromatic regions. The majority of breakpoints in JTs reported in the literature occurred in the centromere or pericentromeric regions, regions rich in DNA repeats, of both the donor and recipient chromosomes (Padilla-Nash et al., 2001; Roschke et al., 2005; Sawyer et al., 2005). More than half of JT breakpoints in our cell lines were not located in these regions (Table 4). Eight cell lines contained JTs and neither donor nor recipient chromosomes exhibited recurrent breakpoints from one cell line to another; our aCGH studies confirmed that JTs within the same cell line share the same breakpoint in the donor. With respect to their consequences, JTs may relocate genes important for tumorigenesis and duplicate the proximal nonhomologous chromosome segments to which they become translocated resulting in a genomic imbalance (Padilla-Nash et al., 2001; Sawyer et al., 2005). The majority of JTs (14/19) in our cell lines were in fact associated with chromosomal gain as shown by aCGH (Table 4).

In total, the results reported here of SKY, FISH, and CGH, combined with the extensive aCGH and gene expression profiling (Camps et al., 2009), 15 CRC cell lines reveal the absence of particular breakpoints leading to recurrent fusions of specific chromosomal bands or genes, but rather resulting in the alteration of genomic copy number. Thus, copy number alterations appear to be the major mechanism for transcriptional deregulation of cancer genes in CRC. The study of chromosomal alterations by SKY served as a critical foundation for interpreting the complex genomic and transcriptomic data obtained by microarray and for the elucidation of mechanisms involved in CRC initiation and progression.

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REFERENCES

Abdel-Rahman WM, Katsura K, Rens W, Gorman PA, Sheer D, Bicknell D, Bodmer WF, Arends MJ, Wyllie AH, Edwards PAW. 2001. Spectral karyotyping suggests additional subsets of colorectal cancers characterized by pattern of chromosome rearrangement. Proc Natl Acad Sci USA 98:2538–2543.

Bardi G, Parada LA, Bomme L, Pandis N, Willén R, Johansson B, Jeppsson B, Beroukas K, Heim S, Mitelman F. 1997. Cytogenetic comparisons of synchronous carcinomas and polyps in patients with colorectal cancer. Br J Cancer 76:765–769.

Baudis M. 2008. Progenetix molecular cytogenetic online database. (2000–2008). Available at: http://www.progenetix.net.

Bosco G, Haber JE. 1998. Chromosome break-induced DNA replication leads to nonreciprocal translocations and telomere capture. Genetics 150:1037–1047.

Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, Sedivy JM, Kinzler KW, Vogelstein B. 1998. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science 282:1497–1501.

Camps J, Morales C, Prat E, Ribas M, Capellà G, Egozcue J, Peinado MA, Miró R. 2004a. Genetic evolution in colon cancer KM12 cells and metastatic derivates. Int J Cancer 110: 869–874.

Camps J, Mrasek K, Prat E, Weise A, Starke H, Egozcue J, Miró R, Liehr T. 2004b. Molecular cytogenetic characterisation of the colorectal cancer cell line SW480. Oncol Rep 11:1215–1218.

Camps J, Grade M, Nguyen QT, Hörmann P, Becker S, Hummon AB, Rodriguez V, Chandrasekharappa S, Chen Y, Difilippantonio MJ, Becker H, Ghadimi BM, Ried T. 2008. Chromosomal breakpoints in primary colon cancer cluster at sites of structural variants in the genome. Cancer Res 68:1284–1295.

Camps J, Nguyen QT, Padilla-Nash HM, Knutsen T, McNeil NE, Wangsa D, Hummon AB, Grade M, Ried T, Difilippantonio MJ. 2009. Integrative genomics reveals mechanisms of copy number alterations responsible for transcriptional deregulation in colorectal cancer. Genes Chromosomes Cancer 48:1002–1017.

Coquelle A, Pipiras E, Toledo F, Buttin G, Debatisse M. 1997. Expression of fragile sites triggers intrachromosomal mammalian gene amplification and sets boundaries to early amplicons. Cell 89:215–225.

Difilippantonio MJ, Petersen S, Chen HT, Johnson R, Jasin M, Kanaar R, Ried T, Nussenzweig A. 2002. Evidence for replicative repair of DNA double-strand breaks leading to oncogenic translocation and gene amplification. J Exp Med 196:469–480.

du Manoir S, Speicher MR, Joos S, Schröck E, Popp S, Döhner H, Kovacs G, Robert-Nicoud M, Lichter P, Cremer T. 1993. Detection of complete and partial chromosome gains and losses by comparative genomic in situ hybridization. Hum Genet 90:590–610.

Dutrillaux B. 1995. Pathways of chromosome alteration in human epithelial cancers. Adv Cancer Res 67:59–82.

Gagos S, Iliopoulos D, Tseleni-Balafouta S, Agapitos M, Antachopoulos C, Kostakis A, Karayannokos P, Skalkeas G. 1996. Cell senescence and a mechanism of clonal evolution leading to continuous cell proliferation, loss of heterozygosity, and tumor heterogeneity: Studies on two immortal colon cancer cell lines. Cancer Genet Cytogenet 90:157–165.

Gayet J, Zhou X-P, Duval A, Rolland S, Hoang J-M, Cottu P, Hamelin R. 2001. Extensive characterization of genetic alterations in a series of human colorectal cancer cell lines. Oncogene 20:5025–5032.

Ghadimi BM, Sackett DL, Difilippantonio MJ, Schröck E, Neumann T, Jauho A, Auer G, Ried T. 2000. Centrosome

amplification and instability occurs exclusively in aneuploid, but not in diploid colorectal cancer cell lines, and correlates with numerical chromosomal aberrations. Genes Chromosomes Cancer 27:183–190.

ISCN. 2005. An International System for Human Cytogenetic Nomenclature. Shaffer LS, Tommerup N, editors. Basel: S. Karger. 130 pp.

Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. 2008. Cancer statistics, 2008. CA Cancer J Clin 58:71–96.

Kawai K, Viars C, Arden K, Tarin D, Urquidi V, Goodison S. 2002. Comprehensive karyotyping of the HT-29 colon adenocarcinoma cell line. Genes Chromsomes Cancer 34:1–8.

Klein CA. 2009. Parallel progression of primary tumours and metastases. Nat Rev Cancer 9:302–312.

Kleivi K, Teixeira MR, Eknæs M, Diep CB, Jakobsen KS, Hamelin R, Lothe RA. 2004. Genome signatures of colon carcinoma cell lines. Cancer Genet Cytogenet 155:119–131.

Knutsen T, Gobu V, Knaus R, Padilla-Nash H, Augustus M, Strausberg RL, Kirsch IR, Sirotkin K, Ried T. 2005. The interactive online SKY/M-FISH & CGH Database and the Entrez Cancer Chromosomes search database: Linkage of chromosomal aberrations with the genome sequence. Genes Chromosomes Cancer 44:52–64.

Kuechler A, Weise A, Michel S, Schaeferhenrich A, Pool-Zobel BL, Claussen U, Liehr T. 2003. Precise breakpoint characterization of the colon adenocarcinoma cell line HT-29 clone 19A by means of 24-color fluorescence in situ hybridization and multicolor banding. Genes Chromosomes Cancer 36:207–210.

Lengauer C, Kinzler KW, Vogelstein B. 1997. Genetic instability in colorectal cancers. Nature 386:623–627.

Lejeune J, Maunoury C, Prieur M, Van den Akker J. 1979. Translocation sauteuse (5p;15q), (8q;15q), (12q;15q). Ann Genet 22:210–213.

Maser RS, DePinho RA. 2002. Connecting chromosomes, crisis, and cancer. Science 297:565–569.

Melcher R, Koehler S, Steinlein C, Schmid M, Mueller CR, Luehrs H, Menzel T, Scheppach W, Moerk H, Scheurlen M, Koehrle J, Al-Taie O. 2002. Spectral karyotype analysis of colon cancer cell lines of the tumor suppressor and mutator pathway. Cytogenet Genome Res 98:22–28.

Melcher R, Steinlein C, Feichtinger W, Müller CR, Menzel T, Lührs H, Scheppach W, Schmid M. 2000. Spectral karyotyping of the human colon cancer cell lines SW480 and SW620. Cytogenet Cell Genet 88:145–152.

Meltzer PS, Guan XY, Trent JM. 1993. Telomere capture stabilizes chromosome breakage. Nat Genet 4:252–255.

Mertens F, Johansson B, Mitelman F. 1994. Isochromosomes in neoplasia. Genes Chromosomes Cancer 10:221–230.

Mitelman F, Johansson B, Mertens F, editors. 2008. Mitelman Database of Chromosome Aberrations in Cancer. Available at: http://cgap.nci.nih.gov/Chromosomes/Mitelman.

Morrow DM, Connelly C, Hieter P. 1997. "Break copy" duplication: A model for chromosome fragment formation in *Saccharomyces cerevisiae*. Genetics 147:371–382.

Padilla-Nash H, Heselmeyer-Haddad K, Wangsa D, Zhang H, Ghadimi BM, Macville M, Augustus M, Schröck E, Hilgenfeld E, Ried T. 2001. Jumping translocations are common in solid tumor cell lines and result in recurrent fusions of whole chromosome arms. Genes Chromosomes Cancer 30:349–363.

Ried T, Knutzen R, Steinbeck R, Blegen H, Schröck E, Heselmeyer K, du Manoir S, Auer G. 1996. Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumors. Genes Chromosomes Cancer 15:234–245.

Roschke AV, Tonon G, Gehlhaus KS, McTyre N, Bussey KJ, Lababidi S, Scudiero DA, Weinstein JN, Kirsch IR. 2003. Karyotypic complexity of the NCI-60 drug-screening panel. Cancer Res 63:8634–8647.

Rowan AJ, Lamlum H, Ilyas M, Wheeler J, Straub J, Papadopoulou A, Bicknell D, Bodmer WF, Tomlinson IPM. 2000. *APC* mutations in sporadic colorectal tumors: A mutational "hotspot" and interdependence of the "two hits." Proc Natl Acad Sci USA 97:3352–3357.

Sawyer JR, Tricot G, Lukacs JL, Binz RL, Tian E, Barlogie B, Shaughnessy J, Jr. 2005. Genomic instability in multiple myeloma: Evidence for jumping segmental duplications of chromosome arm 1q. Genes Chromosomes Cancer 42:95–106.

Schimke RT. 1982. Gene Amplification. Plainview, NY: Cold Spring Harbor Laboratory Press. 339 pp.

Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T. 1996. Multicolor spectral karyotyping of human chromosomes. Science 273:494–497.

Signon L, Malkova A, Naylor ML, Klein H, Haber JE. 2001. Genetic requirements for RAD51- and RAD54-independent break-induced replication repair of a chromosomal double-strand break. Mol Cell Biol 21:2048–2056.

Smith KA, Agarwal ML, Chernov MV, Chernova OB, Deguchi Y, Ishizaka Y, Patterson TE, Poupon MF, Stark GR. 1995. Regulation and mechanisms of gene amplification. Philos Trans R Soc Lond B Biol Sci 347:49–56.

Tsushimi T, Noshima S, Oga A, Esato K, Sasaki K. 2001. DNA amplification and chromosomal translocations are accompanied by chromosomal instability: Analysis of seven human colon cancer cell lines by comparative genomic hybridization and spectral karyotyping. Cancer Genet Cytogenet 126:34–38.